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Laboratory Culture and Life History of *Trichosolen* (=*Pseudobryopsis*) *myura* (J. Agardh) Taylor from Italy

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The morphology and life history of *Trichosolen myura* (J. Agardh) Taylor (Syn. *Pseudobryopsis myura* Berthold) are redescribed, based on material from Italy. Individual thalli are monoecious, with male and female gametes in separate gametangia. Zygotes produced from gametic fusion develop into prostrate filaments with branches and irregularly spaced constrictions. These filaments produce new *Trichosolen* thalli directly. The life history of the alga from Italy is identical with that from Syria (Mayhoub 1974). Herbarium specimens of *T. myura* collected in Japan by Yendo (1915) have been examined and are reassigned to *Bryopsis*.

Introduction

Trichosolen myura (J. Agardh) Taylor was originally described by J. Agardh (1842) under the name of *Bryopsis myura*, and was based on specimens collected in Italy. The type locality was not specified and only a brief morphological description was given. The species was later transferred to *Pseudobryopsis* Berthold in Oltmanns (1904), but *Trichosolen* Montagne (1861) is now recognized as having priority (Taylor 1962). Feldmann (1937, 1969) provided details of the morphology and reproduction of *T. myura* on the basis of the collections at Banyuls on the Mediterranean coast of France. Mayhoub (1974) has also studied the life history of *T. myura* but on specimens collected in Syria. On the basis of these publications it appears that there is a difference in size of gametangia between the specimens from France and Syria. The former has relatively small gametangia. In this paper we describe the morphology and life history of *T. myura* collected in Italy.

Materials and Methods

The specimens used in the present study were collected at Ischia Island near Naples (July 2, 1987) and at Maria la Scola, Sicily Island (July 8, 1987) in Italy by one of us, M. C. They were growing on rocks several meters below low tide. The specimens for morphological observation were preserved in formalin seawater, while living material for laboratory culture was put into plastic bottles with natural seawater and brought back to the laboratory. The unialgal culture was started from excised apical portions of main axes and ramuli. All cultures were grown in ES medium (Provasoli 1966) at 23°C and under a light regime of 18h: 6h (light: dark), with light intensity of 3,000–4,000 lux provided by cool white fluorescent lamps. Under these conditions, the thalli grew well and produced gametes. The filamentous germlings resulting from the zygotes were cultured at five different temperatures: 15°C, 18°C, 21°C, 24°C and 27°C. Observations of nuclei were made using the fluoro-

chrome DAPI (Coleman 1979). In addition, specimens in Yendo's collection at the University Museum, University of Tokyo (TI) (now deposited in the Hokkaido University Herbarium) were examined.

Observations

Morphology

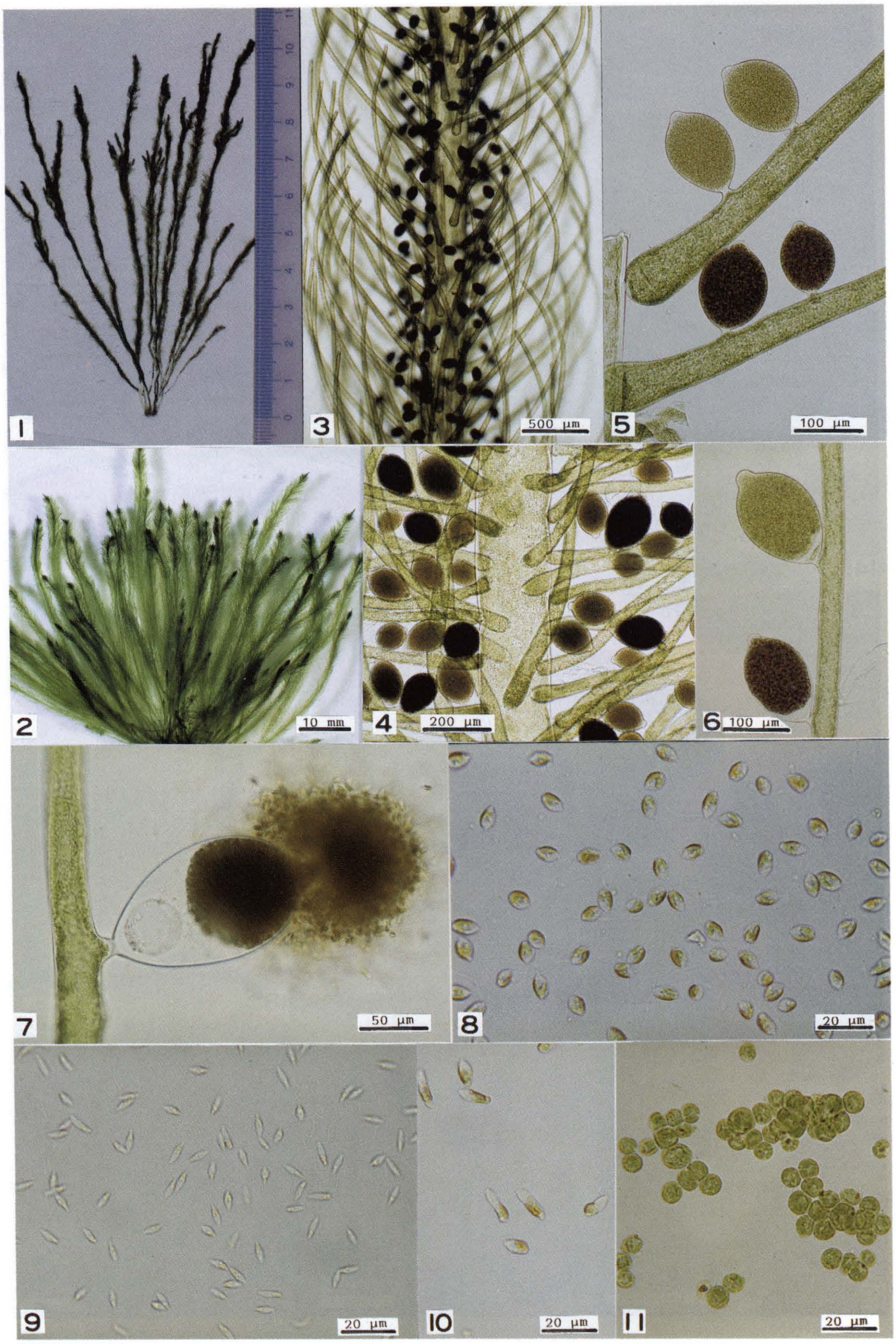
The specimens collected at Ischia Island and Sicily Island are similar to each other in their gross morphology. Thalli are clustered with the numerous main axes originating from a common rhizoidal base (Fig. 1). Main axes are up to 120 mm long and 400–1,000 μm wide. Relatively short main axes are always simple, but longer axes are generally branched subdichotomously or laterally. Axes and branches have numerous ramuli except on the lower part. Ramuli are 2–3 mm long, 20–40 μm wide, soft, and arranged around the branches. Gametangia are ovoid in shape, 100–180 μm long and 65–110 μm wide, and each has a papillum at the tip. They are produced adaxially near the base of ramuli, usually singly, but there are sometimes two or three per ramulus. Chloroplasts are very small, 2–4 μm long and about 2 μm wide, with no pyrenoid.

Life history

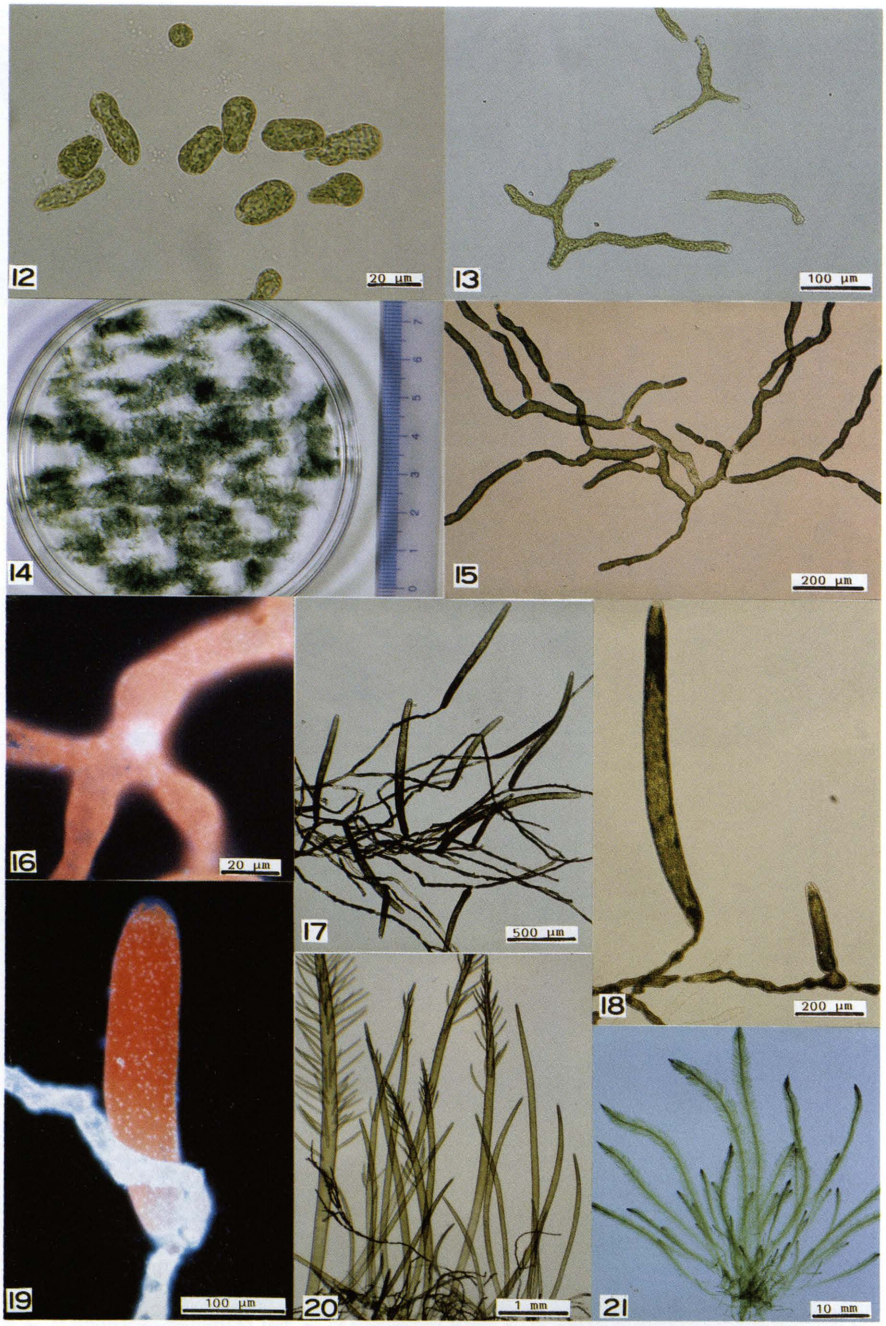
In culture, apical portions grew into erect main axes with ramuli and associated rhizoidal filaments. Later, many erect axes developed from rhizoidal filaments and those produced ramuli in the upper parts. After about a month, the cultured thalli had a clustered appearance (Fig. 2). Many gametangia were produced adaxially, near the base of the ramuli (Fig. 3). The alga was monoecious. Male and female gametangia differ from each other in color: male gametangia were light yellowish-green, while females were dark brownish-green (Fig. 4). Generally gametangia of the same sex were produced on single ramuli (Fig. 5) but rarely the two sexes occurred together (Fig. 6). At the beginning of a light period, the papillum ruptured and gametes were liberated through the aperture. Male gametes were liberated as a jet cloud, as reported in *Trichosolen hainanensis* (Kobara and Chihara 1978a). Female gametes were also liberated rapidly (Fig. 7). When female gametes matured in the gametangia of healthy thalli, they swam about immediately after liberation, without staying outside the gametangium. Sometimes, the liberated female gametes stayed as a mass at the tip of gametangium for one or two minutes before swimming away, as reported by Feldmann (1969) in *T. myura*. Male and female gametes possessed two equal anterior flagella. Female gametes were 7–10 μm long and 4–5 μm wide, and generally possessed several green chloroplasts and a red eyespot (Fig. 8). Male gametes were 4–5 μm long and about 2 μm wide, with one pale green chloroplast and no eye spot (Fig. 9). When male and female gametes met, conjugation took place immediately, resulting in the formation of zygotes (Fig. 10). The zygotes remained motile for about one hour before attaching to the bottom of the culture vessel (Fig. 11). Within three days, zygotes increased in volume, with the formation of a vacuole in the cells. After seven days, they began to germinate (Fig. 12). One week later, the microscopic filament produced one to several lateral branches and irregular constrictions (Fig. 13). After a further six weeks, the much branched filamentous germlings were to 5 mm long and 25–50 μm in diameter (Figs. 14, 15). They had a single nucleus, as already reported by Neumann (1970) (Fig. 16). The germlings attached on oyster shells and corals did not penetrate the substrates.

In order to examine the possibility of parthenogenesis, male and female gametangia were excised from parent thalli and isolated. Unfused male and female gametes did not develop.

After three months growth at temperatures of 21°C and 24°C, the prostrate filaments produced erect filaments directly (Figs. 17, 18). These were produced mostly from the terminal ends and were thicker than



Figs. 1–11. *Trichosolen myura*. 1: Specimens collected at Maria la Scola, Sicily Island (July 8, 1987). 2: Thallus in culture developed from an apical piece of axis or branches, after 4 weeks. 3: Portion of the thallus, showing gametangia produced adaxially near the base of ramuli. 4: Male (light yellowish-green) and female gametangia (dark brownish-green) produced on a thallus. 5: two male gametangia (upper) and two female gametangia (lower) produced on separate ramuli. 6: Male gametangium (upper) and female gametangium (lower) produced on a single ramulus. 7: Female gametangium, liberating female gametes. 8: Female gametes. 9: Male gametes. 10: Zygotes. 11: Zygotes attached to the substratum.



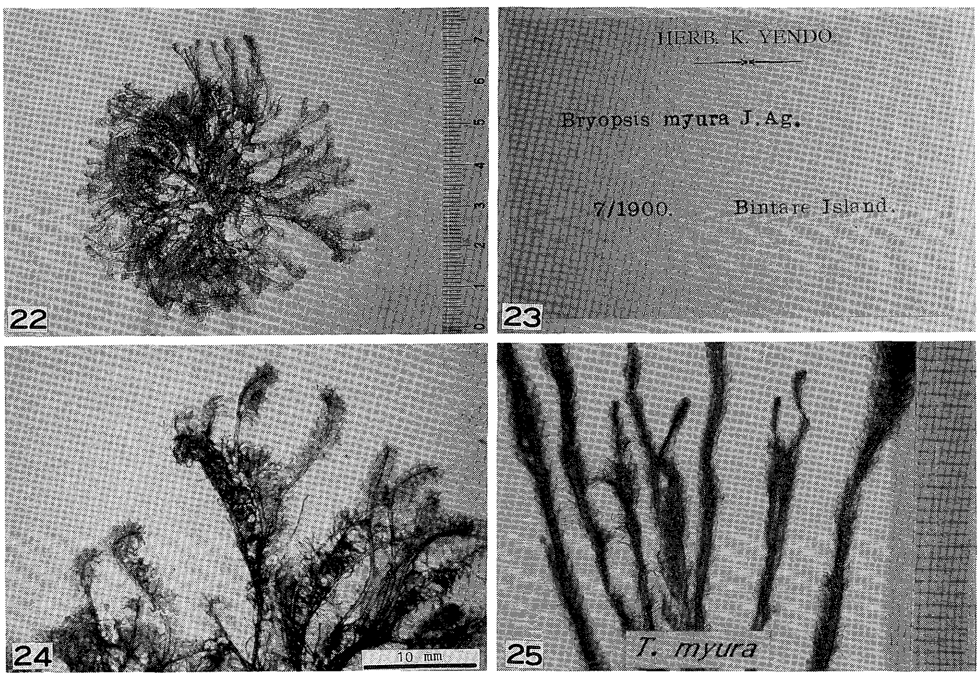
Figs. 12–21. *Trichosolen myura*. 12: Germinating zygotes. 13: Young germling developed from the zygote, with branches. 14: Two month old germlings. 15: Two month old germling with several branches and irregular constrictions. 16: Filamentous germling containing single nucleus. 17–18: Erect filaments developed directly from a filamentous germling. 19: Erect filament containing numerous small nuclei. 20: Erect filament with ramuli. 21: Typical *Trichosolen* thallus developed from the filamentous germling.

the prostrate filaments. One to twelve erect filaments were produced from each germling. Some of the protoplasm of the prostrate filament appeared to move into the erect filaments and, as a result, a part of the prostrate filament became pale green or transparent. At this stage, both the erect thalli and prostrate filament contained numerous small nuclei (Fig. 19). The subsequent formation of a stephanokont zoospore as reported in *T. hainanensis* (Kobara and Chihara 1978b; Okuda et al. 1979) was not observed. The erect filaments grew rapidly and produced many ramuli around the upper part of the axes (Fig. 20), and rhizoidal filaments at the base. Later, these rhizoidal filaments produced several more erect axes. *Trichosolen* thalli as found in nature developed within four months of fertilization (Fig. 21), and these produced gametangia.

In contrast three month old filamentous germlings cultured at 15°C and 18°C did not produce erect filaments. At the higher temperature of 27°C, only a few erect filaments were produced among about a hundred of filamentous germlings, but the production of gametangia was not depressed.

Examination of Japanese specimens of* Trichosolen myura *from the Herbarium of Yendo

In the University Museum, University of Tokyo (TI), in which Yendo's collection was deposited, we found two herbarium specimens named as *Bryopsis myura*, a basionym of *Trichosolen myura*. One of these is shown in Fig. 22 with its label in Fig. 23. Another specimen with similar morphology had the name *Bryopsis myura* written in pencil but it was unlabelled. The gross morphology of the specimens in Yendo's herbarium is clearly different from that of *Trichosolen myura* collected in Italy (Figs. 24, 25). Yendo's specimens had relatively thick ramuli (Fig. 24), and did not exhibit the specialized gametangia,



Figs. 22–24. Yendo's herbarium specimen labelled *Bryopsis myura* from Bintare Island. 25. Portion of an herbarium specimen of *Trichosolen myura* (Sicily Island), at the same magnification as fig. 24.

which are characteristic of *Trichosolen*. In contrast the relatively thin ramuli of *Trichosolen* are arranged densely, and the specialized gametangia can be seen using a dissecting microscope.

Discussion

The morphological features of the specimens collected in Italy agree well with those of *Trichosolen myura* from France (Feldmann 1937, 1969), except for the size of gametangia. In the specimen from Banylus in France, they were reported to be in the range 110–120 μm long and 70–90 μm wide (Feldmann 1937). In the specimen from Syria, they were recorded as 120–220 μm long and 70–100 μm wide (Mayhoub 1974). In our specimens from Italy, gametangia in field collections were 100–180 μm long and 65–100 μm wide, and in culture 90–200 μm long and 65–90 μm wide. These values overlap with those of both French and Syrian specimens.

The life history of *Trichosolen myura* from Italy is fundamentally identical with that reported in Syrian material (Mayhoub 1974). Mayhoub (1974) pointed out the morphological and cytological similarities between filamentous germlings of *T. myura* and *Ostreobium queketii* Bornet et Flahault. *Ostreobium* is a siphonous green alga which penetrates the calcium carbonate substrates. Our experiments show that the filamentous germlings of *T. myura* do not penetrate oyster shells and corals: neither do the germlings of *T. hainanensis* (Kobara and Chihara 1978a). These observations support the Kornmann and Sahling's (1981) view that the two algae are different taxa. Kornmann and Sahling (1981) showed that *O. quekettii* produces quadriflagellate zoospores in the sporangia.

There are similarities between the life histories of *Trichosolen* and *Bryopsis* (Kobara and Chihara 1978b). The life history of *Trichosolen myura* is similar to that of the "Zeeland type" of *Bryopsis plumosa*, whereas that of *T. hainanensis* is "Roscoff type" (Rietema 1969, 1975). Huizing and Rietema (1975, 1979) studied the cell wall constituents of some siphonous green algae and concluded that *Trichosolen* and *Bryopsis* are not closely related. They recorded the cell wall of both the macrothallus and the filamentous microthallus of *T. myura* as consisting mainly of mannan. In *Bryopsis* the cell wall of macrothalli was mainly xylan whereas in the filamentous microthalli it was mainly mannan (see also Chihara et al. 1982). Our observations are at variance with this conclusion. We have examined the constituents of *T. hainanensis* (Chihara et al. 1982) and *T. myura* (Chihara et al. 1988) and in both cases the macrothallus walls were mainly xylan while those of filamentous microthalli were mainly mannan. Our results on the cell wall constituents and the life histories of *Trichosolen* indicate that *Trichosolen* and *Bryopsis* are closely related. The cell wall staining reaction with chlor-zinc-iodine was negative in *Trichosolen* macrothalli, whereas it is positive in *Bryopsis*, as Huizing and Rietema (1975) have already reported. We have no explanation for this discrepancy but note that the cell wall of *Caulerpa* does not stain with chlor-zinc-iodine, even though the main constituent is xylan (Iriki et al. 1960; Miwa et al. 1961).

Trichosolen myura was recorded from Japan by Yendo (1915) under the name of *Bryopsis myura* J. Agardh, but he did not include any morphological description. The locality given was "Hyuga" (Miyazaki-ken). Later, Okamura (1936) recorded *T. myura* in his "Nippon Kaiso-shi" under the name of *Pseudobryopsis myura* (J. Agardh) Berthold, and gave it the Japanese name "Nise-hanemo". However, this record was only a citation of Yendo's record. As far as we know, there is no other published record of *T. myura* from Japan. In the examination of Yendo's herbarium, two sheets of *T. myura* were found. They were collected at Bintare Island (the local inhabitants call this Bindare Island), Kusima-shi, Miyazaki-ken. Probably, *T. myura* from Japan was described on the

basis of these specimens because the island is located in Hyuga (Miyazaki Prefecture). The thick ramuli and absence of specialized gametangia of the specimens indicate that they belong to the genus *Bryopsis*.

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千原光雄，高原隆明：**イタリア産ニセハネモの培養と生活史**

イタリアのナポリ沖にあるイスキア島とイタリア半島南西部にあるシシリー島で採集したニセハネモ ***Trichosolen myura*** (= ***Pseudobryopsis myura***) の形態と，培養による生活史の研究結果を報告した．イタリア産のニセハネモの配偶子嚢は，フランス産のニセハネモのそれよりもはるかに大きい．ニセハネモは雌雄同株で，雌雄の配偶子はそれぞれ別々の配偶子嚢内に生じる．雌雄の配偶子は接合して小さな匍匐糸状体に発達する．糸状体はまばらに分枝し，細胞糸の所々にくびれをもつ．

24℃と21℃の温度条件下において，糸状体から直接的にニセハネモの藻体が発達する．この結果はMayhoub (1974) が報告したシリア産のニセハネモの研究結果と一致する．ニセハネモ属の生活史と細胞壁構成糖から判断すると，ニセハネモ属とハネモ属は極めて近縁であると思われる．日本においてはYendo (1915) が日向からニセハネモの生育を記録している．しかし，宮崎県串間沖の饗垂島から遠藤によって採集された標本にはニセハネモ属特有の配偶子嚢が全くみられず，しかも小羽枝がニセハネモ属のそれよりもはるかに太い．その標本はハネモ属 *Bryopsis* の一種と思われる．